The neuropeptide cart3 enhances finfold regeneration in zebrafish

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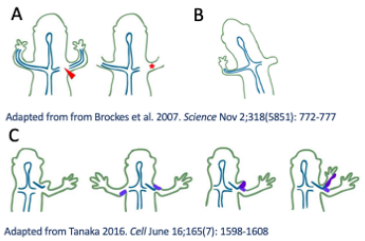
Overview

- Nerve signals are a necessary component of limb regeneration
- Neuropeptide Cart3 is expressed in sensory neurons located within the finfold of larval zebrafish
- Upon amputation, Cart3 is upregulated
- Finfold regeneration is increased by overexpressing Cart3

Regeneration Nerve Dependence

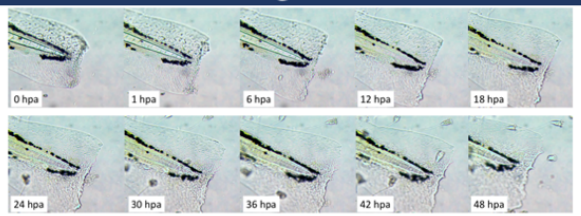


Adapted from from Brockes et al. 2007. *Science* Nov 2;318(5851): 772-777

Adapted from Tanaka 2016. *Cell* June 16;165(7): 1598-1608

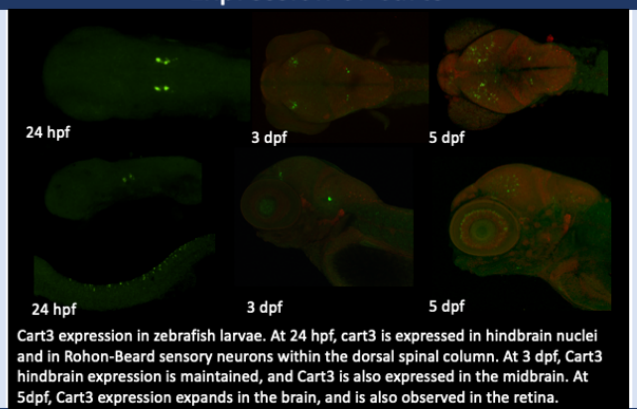
A. After 7 days the salamander is denervated and amputated. **B.** Regeneration only takes place in the innervated limb, indicating that intact nerves are necessary for regeneration. **C.** Nerve deviation results in an ectopic blastema, causing the growth of an accessory limb. This study demonstrates that nerve-derived signals can stimulate limb growth.

Finfold Regeneration



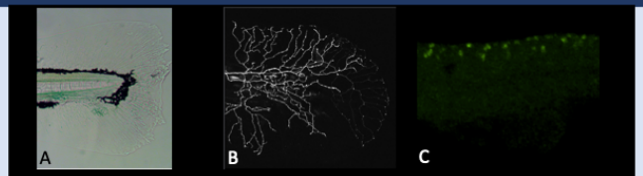
Regeneration spanning 48 hours post amputation

Expression of Cart3



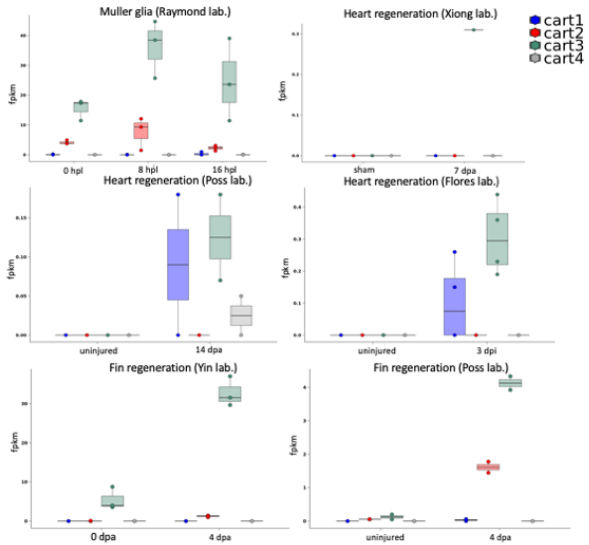
Cart3 expression in zebrafish larvae. At 24 hpf, cart3 is expressed in hindbrain nuclei and in Rohon-Beard sensory neurons within the dorsal spinal column. At 3 dpf, Cart3 hindbrain expression is maintained, and Cart3 is also expressed in the midbrain. At 5dpf, Cart3 expression expands in the brain, and is also observed in the retina.

Innervation of Larval Finfold



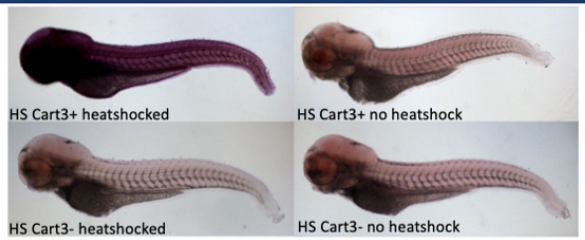
A. Zebrafish caudal finfold at 2 dpf. **B.** Presence of sensory neurons innervating the finfold at 3dpf. **C.** Cart3 expression within sensory neurons of the trunk and tail at 24 hpf.

Cart3 is Upregulated after Finfold Regeneration



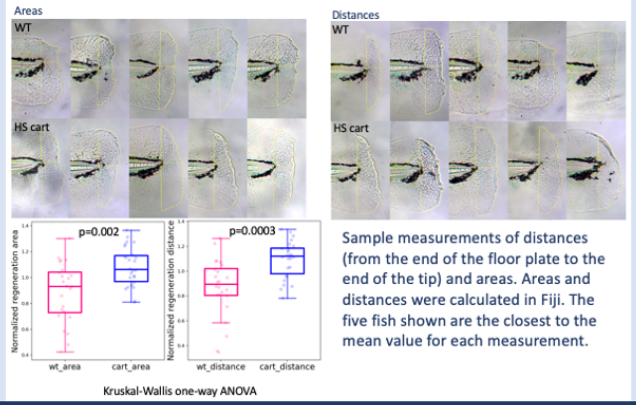
Cart3 expression is induced after tissue damage and remains upregulated during regeneration of numerous tissues. Data taken from http://www.zfregeneration.org

Inducing Cart3 Expression in a Stable Transgenic Line



Larvae were heatshocked at 37 °C for one hour at 48 hpf, and fixed at 51 hpf. Larvae were genotyped by PCR after in situ hybridization.

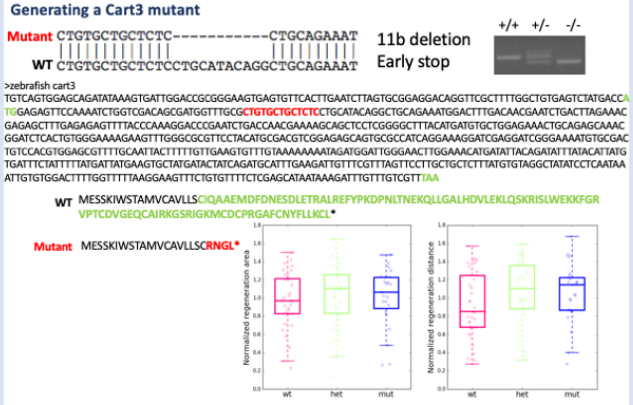
Cart3 Overexpression Enhances Finfold Regeneration



Kruskal-Wallis one-way ANOVA

Sample measurements of distances (from the end of the floor plate to the end of the tip) and areas. Areas and distances were calculated in Fiji. The five fish shown are the closest to the mean value for each measurement.

Cart3 not Required for Finfold Regeneration



Future Directions

- Quantify Cart3-mediated changes cell proliferation within the blastema of regenerating fin folds
- Examine expression of markers of wound epithelium and blastema in Cart3 mutants and Cart3-overexpressing larvae
- Quantify Cart expression in larvae in which leptinb expression has been induced
- Generate mutants for genes that act downstream of leptinb and cart in bone remodeling

Acknowledgements

Special thanks to Madison Chlebowski for all her support and teaching throughout the year. I would also like to thank Laura Bechtler, and the Ithaca College School of Humanities and Sciences for funding this research.